REMARKS / ARGUMENTS

The office action dated August 5, 2010 has been carefully considered. It is believed that the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

At the outset, we would like to thank the Examiner and his Supervisor for the courtesies extended during the telephone discussion on October 7, 2010 and to the Examiner for the follow up telephone discussion of October 26, 2010.

35 USC 103(a)

The Examiner rejected claims 1, 32 and 39-40 under 35 USC 103(a) as being unpatentable over US Patent Publication No. 2004/0142488 to Gierde et al. ("Gierde") in view of US Patent Publication No. 7,379,820 to Sukits et al. ("Sukits"). The Examiner alleges that Gierde describes a method for performing affinity chromatography, in which an affinity molecule fixed on a column captures a biomolecule. The Examiner particularly points to paragraphs [0195-0201] as teaching that the biomolecule can be a multiprotein complex and that one or more constituents of the multi-protein complex can be recovered. In the response dated May 19, 2010, we submitted that this section of Gierde is not citable against the present application. Gierde was published on July 22, 2004 and filed on January 8, 2004. The present application has an international filing date of July 30, 2004 and claims priority dating back to US Provisional 60/494,811 filed on August 14, 2003, which predates the Gierde application. Gierde also claims priority to other previously filed applications, US 10/620,155 filed July 14, 2003, US 60/465,606 filed on April 25, 2003 and US 60/396,595 filed July 15, 2002. Applicants reviewed these three Gierde priority documents and respectfully submitted that these three documents do not provide support for paragraphs [195-202] of the presently cited Gierde application. Thus, Applicants respectfully submitted that paragraphs [195-201] were filed and published after the present application's first priority document, US 60/494,811 and were not citable against the present application. In response to the above arguments, at page 14 of the office action the Examiner pointed to the Gierde priority document US Provisional Application No. 60/396,595 and cites pages 72-73, corresponding to Affinity Chromatography: Principles and Methods, page 42 in the section entitled "Recombinant Fusion Proteins". According to the Examiner this section

provides sufficient support for Gierde's description of multi-complex proteins separated using an affinity column. Applicants respectfully disagree for the reasons that follow.

The cited section of the '595 Gierde priority application merely discloses that purification of recombinant proteins can often be simplified by incorporating a tag of known size into the protein and goes on to describe GST tags and histidine tags. The resulting protein is a fusion protein of the tag and the recombinant protein. We respectfully submit that this section does not describe multi-complex proteins as recited in the present claims, i.e. comprising a first ligand that associates *in vivo* with a second ligand. In a fusion protein, the protein is translated as a fused protein, would be considered by a person skilled in the art as a single protein and thus does not equate to multi-complex proteins that associate *in vivo*. In view of the foregoing, we submit that the '595 Gierde priority document does not provide support for multi-complex proteins nor does it provide support for separating multi-complex proteins using an affinity column and thus the sections of Gierde [0195-0202] relied upon by the Examiner are not citable against the present application.

The deficiencies of Gierde are not remedied by Sukits. Sukits merely discloses a series of protein pairs, e.g. TRADD and RIP, FAS and FADD, and TNFR-1 and TRADD that associate together through electrostatic interactions.

In view of the above, Applicants respectfully submit that claims 1, 32 and 39-40 are inventive over Gierde in view of Sukits.

The Examiner rejected claim 37 under 35 USC 103(a) as being unpatentable over Gierde in view of Sukits as applied to claims 1 and 34 above, and further in view of US Patent Application Publication NO. 2003/0229212 to Fahrner et al. ("Fahrner"). According to the Examiner, Fahrner discloses an ion-exchange chromatography as a competition between an ion and a substrate for a molecule of interest. The Examiner is therefore of the opinion that in light of Gierde in view of Sukits and Fahrner, the NaCl competes with the complex to elute a protein in the multi-protein complex, thereby binding to one of the proteins and meeting the claimed limitation. Applicants respectfully disagree for the reasons that follow.

For the reasons cited above, Applicants submit that claims 1 and 34 are inventive over Gierde in view of Sukits. Fahrner merely describes ion exchange chromatography in the background and thus does not correct for the deficiencies of Gierde in view of Sukits.

In view of the above, Applicants respectfully submit that claim 37 is inventive over Gierde in view of Sukits and further in view of Fahrner.

The Examiner rejected claims 1-23, 25-26, 34-35 and 38 under 35 USC 103(a) as being obvious having regard to Rigaut et al. ("Rigaut", Nature Biotechnology (1999) 17:1030-1032) in view of Sukits and Gierde. The Examiner alleges that it would be obvious to modify Rigaut's method to investigate protein-protein interactions in multi-protein complexes using electrostatic elution. According to the Examiner, the person skilled in the art would modify Rigaut's method to include a NaCl elution step on the multi-protein complex and that the person skilled in the art would be motivated to do so based on Gierde's indication that the nature of multi-protein complexes can be analyzed by eluting individual components. Applicants respectfully disagree for the reasons that follow.

As mentioned above, Applicants respectfully submit that as of the priority date of the present application, Gierde did not disclose that the nature of multi-protein complexes can be analyzed by eluting individual components. Such an indication was only added to Gierde after the present application's priority date. In view of the foregoing, the Gierde sections relied upon by the Examiner are not citable against the present application and thus, we respectfully submit that there is no motivation to modify Rigaut's method in view of Sukits.

Although during the tandem affinity purification of Rigaut, it is possible that the TAP-tagged target protein would be isolated along with other proteins, there is no indication that the first and second ligands can associate through electrostatic forces or that they can be separated from each other by decreasing the electrostatic force between them. Rigaut does not teach or suggest that the second ligand (the protein that is immobilized on the affinity matrix) must remain immobilized on the affinity column during the elution of the first ligand. As described in the present application, desorption of the second ligand must be avoided, see paragraph [0354] of the present application. Otherwise, a severe dynamic range problem will be encountered, which will make the identification of the first ligand impossible. See [0005-0015] and [0077- FIG. 21] of the present application. Thus one of ordinary skill in the art following the teachings of

Rigaut would isolate the permanent and transient members of the protein complex, always isolated in different amounts, together and present the results as a strong proof for their interaction/association.

Prior to the present application, a person skilled in the art would not be motivated to disrupt electrostatic interactions from the immobilized multi-protein complex described in Rigaut based on the disclosure of Sukits.

At the top of p15 the Examiner alleges that Sukits discloses disrupting electrostatic interactions between proteins that come together *in vivo*. We respectfully disagree. Sukits never studied *in vivo* formed protein pairs. All the protein pairs that Sukits studied, i.e. RIP DD-TRADD, FAS DD-FADD DD and TNFR-1 DD-TRADD DD, were formed *in vitro*, i.e. artificial complexes created *de novo* during the experiment. Applicants respectfully submit that a person skilled in the art would not expect that two proteins associate *in vivo* in the same way they associate *in vitro*. In addition, the immobilized proteins (second ligand) of Sukits are not present in full length and are not synthesized in their respective endogenous organisms. A person skilled in the art would know that even though the truncated forms of two proteins associate electrostatically *in vitro* and increasing the ionic strength dissociates the complex, it does not follow automatically that the same two proteins associate in the same way *in vivo* and that they can be separated upon salt elution. See for the example of resistance to salt elution of the electrostatic bonds between Rpb1, Rpb2, Rpb5, Rpb6 and Rpb8 (Cramer P, Bushnell D, Kornberg R., Science, 2001, submitted with response dated May 19, 2010). In fact, Sukits states at column 18, lines 30-34 that this *in vitro* formed pair could be disrupted by NaCl titration indicating that "the homotypic interaction is **at least in part** electrostatic." (Emphasis added). Thus, Sukits acknowledges that other interactions may also be present and thus, confirms that the *in vitro* interaction does not necessarily relate to how the two proteins come together *in vivo*.

The Applicants were the first to demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable. Prior to the present inventor's demonstration of the advantages of separating the first ligand from the second ligand that remains immobilized on the column, a person skilled in the art would not be motivated to modify the Rigaut method. Further, since the present claims relate to proteins

associating *in vivo*, Applicants submit that there would be no motivation to combine Rigaut with the *in vitro* disclosure of Sukits.

In addition, the person skilled in the art would not expect to be able to separate transient proteins from permanent protein members of an *in vivo* formed multi-protein complex. Sukits teaches that decreasing the electrostatic forces leads to separation/elution of the protein(s) that are noncovalently bound to the affinity matrix. This is the reason why, before applying high salt elution, Sukits teaches covalent immobilization of one member of the protein pair to the affinity column. See that in Jeong et al., 1999, page 16338, bottom left – "Mutations and Measurements of Binding Affinity" (submitted with the response dated May 19, 2010) one of the members of a protein pair that associate by electrostatic forces is always covalently bound to the affinity column for the purpose of keeping it immobilized during the NaCl titration.

In view of the above, a person of ordinary skill in the art would expect that after immobilizing a protein complex via an affinity tag (following Rigaut), the ionic strength used in Sukits would destroy the non-covalent bond, such as a protein-protein bond, between the affinity column and affinity tag and would result in releasing the entire complex, i.e. second ligand and first ligand. As an example, Sukits demonstrates that 0.3M NaCl releases the protein that is selectively but noncovalently bound to the affinity column. So, one with ordinary skill in the art would expect that even 0.3M NaCl would release the second ligand from the affinity column.

In view of the above, Applicants respectfully submit that claims 1-23, 25-26, 34-35 and 38 are inventive over Rigaut in view of Sukits and Gierde.

The Examiner rejected claim 24 under 35 USC 103(a) as being unpatentable over Rigaut in view of Sukits and Gierde as applied to claims 1 and 22-23 above, and further in view of US Patent No. 5,007,934 to Stone and US Patent No. 5,849,885 to Nuyens et al ("Nuyens"). For the reasons discussed above, claims 1 and 22-23 are inventive over Rigaut in view of Sukits and Gierde. The Examiner cites Stone as disclosing using NaCl or KCl as equivalent salts for removing glycoprotein or proteoglycan associated with collagen through electrostatic interaction and cites Nuyens as disclosing NaCl or KCl as equivalent salts for reducing electrostatic interactions between lactoferrin and other proteins. Thus, neither Stone nor Nuyens correct for the deficiencies discussed above of Rigaut in view of Sukits and Gierde.

The Examiner rejected claims 45 and 46 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sukits and Gierde as applied to claim 1 above, and further in view of Patent 6,610,508 to Hentze et al. and evidenced by US Patent No. 5,753,225 to Clary et al. For the reasons discussed above, claim 1 is inventive over Rigaut in view of Sukits and Gierde. According to the Examiner, Clary et al. discloses receptor-ligand complexes as reversible electrostatic attractions, and Hentze et al. describes identifying protein-protein interactions in order to detect disease states, including Alzheimer's disease. Thus, the deficiencies of Rigaut, Sukits and Gierde are not remedied by either Hentze et al. or Clary et al.

In view of the above, Applicants respectfully submit that the present claims are inventive over Rigaut in view of Sukits and Gierde alone or having regard further to Hentze, Stone, or Nuyens.

In view of the foregoing, Applicants respectfully request that the rejections under 35 USC 103(a) be withdrawn.

The Commissioner is hereby authorized to charge any fee (including any claim fee) which may be required to our Deposit Account No. 02-2095.

In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance and early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, he is kindly requested to contact the undersigned by telephone at (416) 957-1678 at his convenience.

Respectfully submitted,

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